Interference in Ionized Calcium Measurements by Heparin Salts

Michael Landt,1,2,5 Glen L. Hortin,1,2,4 Carl H. Smith,1,2 Adrain McClellan,2,3 and Mitchell G. Scott2,3

We determined the suitability of various heparin salts used for anticoagulation of whole-blood specimens for measurement of ionized calcium (iCa), blood gases, and electrolytes. We were particularly interested in a new heparin product containing both zinc and lithium cations (CNLZ heparin), in which the binding sites with greatest affinity for divalent cations are bound with zinc and low-affinity sites with lithium. In initial experiments Li heparin decreased iCa concentrations 0.07 mmol/L at the lowest heparin concentration (3000 units/L) and progressively lowered them at higher concentrations. Zn heparin initially increased iCa concentrations 0.06 mmol/L but progressively lowered them as the heparin concentration was increased. Li heparin interfered even when present in amounts (9 units per 3-mL syringe) minimally effective in preventing coagulation. Use of CNLZ heparin (36 units per 3-mL syringe; Zn 63–78 g/kg of heparin) largely eliminated interference of heparin in iCa measurements. In studies that included the effects of concentration of heparin through partial filling of syringes, specimens anticoagulated with CNLZ heparin compared well with unheparinized controls in measurements of iCa, blood gases, and electrolytes. Blood gases and iCa results on CNLZ heparinized specimens from intensive-care-unit patients also compared well with specimens anticoagulated with a preparation of heparin (EB heparin) in which calcium has been added to balance the calcium-binding capacity. However, the presence of calcium in EB heparin significantly increased measured total calcium concentrations, whereas the new CNLZ heparin did not interfere in total calcium determinations.

Indexing Terms: zinc/lithium/divalent cations/blood gases/anticoagulants

Measurement of ionized calcium (iCa) is increasingly used in instances where total calcium measurements provide an incomplete assessment of physiologic calcium status (1), e.g., in premature infants (2) or those with derangement of protein metabolism (3).6 Continuing improvement in analytical technology has made iCa analysis more reliable and practical for the clinical laboratory (4). One aspect of this improved technology is that small amounts of whole blood can be used as specimen, obviating the need for separation of plasma and facilitating anaerobic handling. Change in specimen pH during transport/processing/analysis, resulting from the evolution of CO₂, significantly decreases iCa (2). Whole-blood specimens for iCa analyses are usually collected in syringes containing heparin, and many hospitals have found it efficient to collect and perform iCa, arterial blood gas, and other electrolyte analyses on the same specimen.

A frequently unrecognized but significant problem in using heparinized whole-blood specimens for iCa determinations is interference from heparin. Heparin binds calcium and thereby reduces the chemical activity of the calcium present in the specimen; the reduced activity is reflected as reduced iCa concentration (5). Several types of heparin-containing syringes have been developed to minimize interference by (a) reducing the heparin content of the syringe to the absolute minimum necessary for anticoagulation (6); (b) using the zinc salt of heparin (7); and (c) titrating the heparin with calcium to “balance” the heparin binding effect (8, 9).

Heparin binding of divalent cations is heterogeneous, reflecting the presence of both high- and low-affinity binding sites (10). Recently, an alternative heparin product, calcium-neutralized lithium zinc heparin (CNLZ heparin), that took into account this heterogeneity was developed: The lithium salt of heparin was treated with sufficient zinc to occupy high-affinity sites, and the lower-affinity binding sites were exchanged back to lithium. The rationale is that calcium binding to heparin occurs at sites with high affinity for divalent ions, and that titration with zinc would preferentially occupy these sites with a nondissociable metal ion. Subsequent exposure of blood specimens to the titrated heparin would not alter iCa concentrations because binding of calcium to heparin would be prevented by occupation of the high-affinity binding sites by zinc. Here we report studies of the optimization of zinc content and a clinical laboratory evaluation of the efficacy of CNLZ heparin.

Materials and Methods
Subjects
Initial studies were conducted with whole-blood specimens collected from healthy adult volunteers. The clinical efficacy study included specimens collected from adult patients following a cardiothoracic operative procedure. This study strictly followed the protocol of the Human Studies Committee of Washington University School of Medicine, and all volunteers and patients provided written informed consent prior to participation.
Syringes and Heparin Preparations

The following types of heparin were tested: CNLZ heparin (trademark Ca³⁺Lyte), containing both zinc and lithium as cations, evaluated as liquid preparations initially and in lyophilized form subsequently in a commercial syringe product, Aspir-Pulse ABG syringe, containing 36 units of heparin (prod. no. 8884–252001; Sherwood Medical Co., St. Louis, MO); "electrolyte-balanced" (EB) heparin, containing calcium, sodium, and potassium heparin in lyophilized form in a commercial syringe product, Smooth-E ABG syringe, containing 120 units of heparin (prod. no. 956-335; Radiometer America, Westlake, OH); Zn heparin, containing zinc as counterion and obtained as a dry powder from Celsus Laboratories (Cincinnati, OH); and Li heparin, containing lithium as counterion and obtained from Scientific Protein Labs (Waunakee, WI). Solutions of Zn heparin, Li heparin, and CNLZ heparin were prepared in highly concentrated stocks (3 × 10⁶ units/L) in water.

Specimens

For studies of the effects of concentration and types of heparin on laboratory analytical measurements, blood was drawn from the antecubital vein of healthy adult volunteers by using a winged infusion set, without the use of a tourniquet. Blood (10–20 mL) was drawn and discarded to minimize the effects of the temporary stasis needed to achieve venipuncture, and then blood was drawn in various volumes into 3.0-mL plain syringes that either contained dry heparin or had 12 μL of various heparin solutions pipetted into the syringe tip dead space with a fine-tip variable-volume pipette. A syringe without anticoagulant, which served as the control, was always drawn last and analyzed immediately (<5 min), before clot formation occurred (9).

For evaluation of the 3-mL syringe containing 36 units of lyophilized CNLZ heparin under anticipated clinical conditions, specimens (2–3 mL) were drawn from patients in a cardiac intensive-care unit through arterial lines surgically placed in the radial artery. Three specimens were collected from each patient with a 3-mL syringe containing 36 units of lyophilized CNLZ heparin, a 3-mL syringe containing 120 units of lyophilized EB heparin, and a plain syringe. The plain syringe specimen was anaerobically transferred via needle to an unheparinized tube (no. 8881-301413; Sherwood Medical). Specimens were transported on ice to the laboratory and analyzed within 30 min. Analysis of some specimens was purposely delayed for up to 2 h to simulate the effects of delay in analysis due to extended transport time and (or) technical problems.

Analyzers

ICA-1 analyzers (Radiometer America) were used to measure iCa, according to the manufacturer’s directions and with reagents supplied by Radiometer America. Blood gas analyses and determinations of sodium and potassium concentrations in whole blood were performed with either BGElectrolyte analyzers (Instrumentation Laboratory, Lexington, MA) or with ABL-4 analyzers (Radiometer America). Total calcium analyses were performed on an Ektachem 700 XR analyzer (Eastman Kodak, Rochester, NY) after separation of plasma or serum by centrifugation at 1100g for 5 min.

Results

In an initial experiment with venous whole-blood specimens collected from volunteers, we compared the effects of Li heparin, Zn heparin, and EB heparin on iCa measurements with those on whole blood drawn without anticoagulant and analyzed immediately, before clotting occurred. iCa values obtained from the latter specimens were regarded as the best estimate of the true iCa concentrations in each volunteer because no anticoagulant was present to bind calcium and immediate analysis eliminated the effects of coagulation (9). All syringes contained 100 units of heparin; this dose was chosen because many commercially available heparin-containing 3-mL syringes contain this amount. Final heparin concentrations varied from 33 000 units/L to 248 000 units/L by varying the draw volume in syringes from 3.0 to 0.4 mL. We evaluated the effects of higher concentrations of heparin that result from smaller draw volumes, since this is a common occurrence in clinical practice. Li heparin significantly decreased iCa measurements at all concentrations, and the highest concentrations decreased apparent iCa concentrations below the lower limit of normal (0.98–1.30 mmol/L) (Fig. 1). Zn heparin increased iCa at low concentrations (33 000–66 000 units/L) but progressively decreased iCa at higher concentrations (Fig. 1). The decreases in iCa

---

Fig. 1. Interference of heparin types in iCa measurement. 3-mL syringes initially contained 100 units of Zn heparin, Li heparin, or EB heparin. Heparin concentrations increased as draw volume decreased. All points represent the average of four determinations, including the initial point for the unheparinized control.

---

566 CLINICAL CHEMISTRY, Vol. 40, No. 4, 1994
caused by higher concentrations of Zn heparin paralleled the Li heparin curve, with an offset of 0.13 mmol/L higher iCa concentration. EB heparin did not alter iCa concentrations in any of the specimens collected over the full range of heparin concentrations (Fig. 1). We also observed that Zn heparin lowered the pH of whole-blood specimens from a mean of 7.39 (± 0.03; n = 4) in the controls to 7.38 (± 0.02) in specimens collected with the lowest concentration (33 000 units/L) of Zn heparin, and pH fell progressively to 7.28 in specimens collected with 248 000 units/L Zn heparin. All pH measurements of specimens containing Zn heparin except that collected with the lowest concentration were statistically different from the control (P <0.05).

To minimize heparin interference in iCa measurements, some manufacturers of commercial heparin-containing syringes reduce the heparin content of their products to the minimum necessary to assure adequate anticoagulation. When the Zn heparin content of 3-mL syringes was reduced from 100 to 30 units and volunteer specimens were collected in syringe volumes of 3.0–0.4 mL, iCa concentrations were modestly but consistently higher than for nonanticoagulated controls at heparin concentrations of 10 000–40 000 units/L (Fig. 2). iCa concentrations decreased at higher Zn heparin concentrations; pH was not decreased at any Zn heparin concentration. We also examined the efficacy of reducing the Li heparin content of syringes; the first experiment involved 3-mL syringes with 30 units of Li heparin and a second experiment involved syringes with 9 units of Li heparin (Fig. 2). In both instances, iCa concentrations were only slightly lower than controls at the lowest heparin concentration, but dropped rapidly and progressively as the heparin concentration increased (draw volume decreased) (Fig. 2), despite the minimal amounts of heparin present. Clotting of specimens was not observed during the period (<1 h) required to complete these analyses.

Because at low heparin concentrations (10 000–50 000 units/L) the effects of Zn heparin and Li heparin on iCa values appeared to be opposite, we evaluated a series of 12 CNLZ heparin preparations with zinc contents of 19.5–127 g/kg dry porcine heparin. As before, 3-mL syringes containing 36 units of the varied preparations of CNLZ heparin were used to draw blood from volunteers at final syringe volumes of 0.4–3.0 mL. Control specimens were collected in plain syringes without anticoagulant and analyzed promptly. The iCa results are presented as a bias plot, in which the unheparinized (control) iCa value was the starting point for experimental values in a given experiment and subsequent points are expressed as net change from the control value in each experiment (Fig. 3); representative curves for six doses are presented. When Zn content exceeded 78 g/kg heparin, modest but sustained increases in iCa concentrations were found at draw volumes of ≤1.5 mL. Zn content of <59 g/kg resulted in modest but reproducible decreases in iCa concentrations in the range of 12 000–48 000 units/L heparin (3.0–0.75-mL draw volume). CNLZ heparin preparations with Zn at 59.4–78.0 g/kg heparin performed best; at 0.75–3.0-mL draw volumes (12 000–48 000 units/L), the normalized iCa values deviated <0.025 mmol/L from the control values (Fig. 3), which was clinically insignificant.

The efficacy of CNLZ heparin [36 units (lyophilized)/syringe: Zn 74.2 g/kg] was further evaluated in a study...
of blood gas, total calcium, and iCa in specimens collected from volunteers. Whole-blood specimens collected without anticoagulant and analyzed immediately served as controls, and specimens collected in syringes containing EB heparin were also drawn for comparison. Blood was drawn into each type of syringe at volumes of 3.0 mL (nominal maximum capacity) and 0.75 mL, regarded as the minimum volume in routine clinical use. Results for iCa in specimens collected in both CNLZ and EB heparin-containing syringes agreed well at both draw volumes with control syringe results (Table 1). iCa results for specimens collected with CNLZ heparin were significantly higher than control draws (\( P = 0.01 \)) for both volumes, but the average difference in paired values was a clinically insignificant 0.014 mmol/L (mean values = 1.23 ± 0.03 mmol/L for both 3.0- and 0.75-mL draw volumes vs 1.22 ± 0.03 mmol/L for controls). Comparison of blood gas measurements (\( P_{O_2}, P_{CO_2}, pH \)) from specimens collected in syringes containing CNLZ or EB heparin with results from controls found no bias with either syringe (Table 2); all \( P \) values were >0.05 and means for each parameter were similar. Total calcium concentrations were also measured in plasma obtained from these specimens because of the potential for EB heparin to artificially increase this measurement (9). Total calcium values from specimens collected with CNLZ heparin agreed well with paired control results (Table 2). Though total calcium was significantly decreased in the 0.75-mL specimens, the average difference between paired specimens was a clinically insignificant 0.07 mmol/L. Total calcium values for specimens collected with EB heparin were significantly higher (\( P < 0.001 \)) at both 3.0 and 0.75 mL than the control syringe results (Table 2). Average differences between paired specimens for total calcium were 0.09 mmol/L higher in 3.0-mL specimens and 0.46 mmol/L higher in 0.75-mL specimens; in the latter, the average total calcium concentration (2.85 mmol/L) exceeded our laboratory normal range (2.2–2.6 mmol/L).

The efficacy of CNLZ heparin (36 units/3-mL syringe, Zn 77.6 g/kg heparin, lyophilized form) was assessed with arterial blood specimens collected from cardiac intensive-care unit patients for iCa, total calcium, electrolytes, and blood gas analyses. A whole-blood specimen was collected in the laboratory’s routinely used syringe containing EB heparin (100 units/3-mL syringe) for comparison of electrolyte and blood gas measurements. The control for iCa and total calcium in this experiment was a concomitantly collected whole-blood specimen that was allowed to clot in a tightly stoppered serum-collection tube. A preliminary experiment comparing iCa measured immediately in whole-blood specimens collected without anticoagulant with iCa in the serum control showed that average iCa values decreased from 1.261 to 1.248 mmol/L, respectively (\( n = 7 \)). From this experiment, we conclude that iCa concentrations in freshly drawn and analyzed arterial specimens will average slightly higher (0.013 mmol/L) than concomitantly collected serum control specimens if the anticoagulant in test syringes introduces no artificial change in iCa concentrations. Specimens collected in syringes containing EB heparin were also compared with the serum controls for iCa and total calcium analyses. With regard to iCa results, both CNLZ heparin and EB heparin specimens were significantly higher than serum controls (CNLZ heparin \( \bar{x} = 1.19 \) mmol/L, EB heparin \( \bar{x} = 1.18 \) mmol/L, serum \( \bar{x} = 1.16 \) mmol/L, \( n = 26 \)) (Table 3) and the average disparity was slightly greater (0.03 and 0.02 mmol/L, respectively) than that predicted by the earlier experiment (0.013 mmol/L). The range of disparity between iCa values in heparinized specimens and paired serum controls was –0.03–0.10 mmol/L, probably reflecting the variable effects of clotting and pH changes on the serum iCa of paired specimens (9). These small differences were clinically insignificant.

In comparisons of blood gas results from CNLZ heparin specimens with results from EB heparin (control) specimens, there were no statistically or clinically significant differences (Table 4): Values for \( P_{O_2} \) averaged 235 vs 241 mmHg (\( P = 0.11 \)); pH measurements averaged 7.43 vs 7.43 (\( P = 0.88 \)); and \( P_{CO_2} \) averaged 36 vs 36 mmHg (\( P = 1.00 \)), respectively. Potassium and sodium determinations were also performed on CNLZ- and EB heparin-treated whole-blood specimens. Average potassium values were nearly identical (4.04 vs 4.03 mmol/L respectively; \( P = 0.38 \)) (Table 4). Average sodium values were also nearly identical (138.0 vs 138.4 mmol/L, respectively), but significantly different (\( P = 0.02 \)) (Table 4).

Total calcium values were compared for plasma obtained from arterial blood specimens collected with either CNLZ heparin or EB heparin vs a concomitantly collected serum specimen (Table 3). Collection of blood with EB heparin statistically and clinically increased total calcium values an average of 0.11 mmol/L (EB heparin \( \bar{x} = 2.20 \) mmol/L, serum \( \bar{x} = 2.09 \) mmol/L, \( P < 0.01 \)). Results for specimens collected with CNLZ heparin closely agreed with paired serum results (CNLZ heparin \( \bar{x} = 2.08 \) mmol/L, serum \( \bar{x} = 2.09 \) mmol/L) but were significantly different (\( P = 0.03 \)).

**Discussion**

The ideal syringe for collection of specimens for iCa analysis would contain sufficient anticoagulant so that the specimen would remain free of clots for a period that

### Table 1. iCa values for blood specimens collected in control and heparin-containing syringes with varied draw volumes.

<table>
<thead>
<tr>
<th>Heparin type</th>
<th>Draw volume, mL</th>
<th>iCa, mmol/L, mean ± SD</th>
<th>Comparison with control syringe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( S_{Pr} )</td>
<td>( P )</td>
</tr>
<tr>
<td>CNLZ</td>
<td>3.0</td>
<td>1.23 ± 0.03</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>1.23 ± 0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>EB</td>
<td>3.0</td>
<td>1.21 ± 0.03</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>1.22 ± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>1.22 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

* \( n = 10 \) for all means and paired comparisons.
Table 2. Blood gas and total calcium measurements of specimens collected in syringes containing CNLZ heparin or EB heparin and those of specimens collected without anticoagulant.

<table>
<thead>
<tr>
<th>Heparin type</th>
<th>Specimen volume, mL</th>
<th>pH Mean ± SD</th>
<th>P&lt;sub&gt;b&lt;/sub&gt;</th>
<th>PO&lt;sub&gt;2&lt;/sub&gt;, mmHg Mean ± SD</th>
<th>P&lt;sub&gt;b&lt;/sub&gt;</th>
<th>PO&lt;sub&gt;2&lt;/sub&gt;, mmHg Mean ± SD</th>
<th>P&lt;sub&gt;b&lt;/sub&gt;</th>
<th>Total calcium, mmol/L Mean ± SD</th>
<th>P&lt;sub&gt;b&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNLZ</td>
<td>3.0</td>
<td>7.38 ± 0.03</td>
<td>0.298</td>
<td>42.4 ± 5.6</td>
<td>0.818</td>
<td>48.5 ± 16.6</td>
<td>0.969</td>
<td>3.25 ± 0.05</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>7.37 ± 0.03</td>
<td>0.052</td>
<td>43.8 ± 6.1</td>
<td>0.246</td>
<td>45.8 ± 19.8</td>
<td>0.692</td>
<td>3.23 ± 0.06</td>
<td>0.043</td>
</tr>
<tr>
<td>EB</td>
<td>3.0</td>
<td>7.39 ± 0.02</td>
<td>0.081</td>
<td>43.2 ± 5.4</td>
<td>0.147</td>
<td>44.3 ± 12.9</td>
<td>0.351</td>
<td>3.48 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>7.38 ± 0.03</td>
<td>0.285</td>
<td>42.5 ± 6.5</td>
<td>0.737</td>
<td>51.8 ± 14.5</td>
<td>0.500</td>
<td>2.85 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>7.39 ± 0.02</td>
<td>0.052</td>
<td>42.2 ± 5.0</td>
<td>0.03</td>
<td>48.7 ± 13.2</td>
<td>0.05</td>
<td>3.28 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 10 for all means and paired comparisons.
<sup>b</sup> Student's t-test was used to calculate P values (P < 0.05 regarded as statistically significant) for test syringes vs paired control (no heparin) values.
<sup>c</sup> n = 4 (only paired control specimens were used for statistical comparison).
<sup>d</sup> n = 7 (only paired control specimens were used for statistical comparison).

Table 3. Comparison of iCa and total calcium values in arterial blood specimens collected from intensive-care-unit patients in syringes containing CNLZ or EB heparin with those from a concomitantly collected serum specimen.

<table>
<thead>
<tr>
<th>Heparin type</th>
<th>iCa</th>
<th>Total calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± Slope Intercept S&lt;sub&gt;yx&lt;/sub&gt; P</td>
<td>Mean± Slope Intercept S&lt;sub&gt;yx&lt;/sub&gt; P</td>
</tr>
<tr>
<td>CNLZ</td>
<td>1.19± 0.98 0.06 0.03 &lt;0.01</td>
<td>2.06± 1.02 −0.06 0.04 &lt;0.03</td>
</tr>
<tr>
<td>EB</td>
<td>1.18± 0.93 0.10 0.03 &lt;0.01</td>
<td>2.20± 1.01 0.07 0.04 &lt;0.01</td>
</tr>
<tr>
<td>Serum</td>
<td>1.16±</td>
<td>2.09±</td>
</tr>
</tbody>
</table>

<sup>a</sup> mmol/L.
<sup>b</sup> n = 26.
<sup>c</sup> n = 22.
<sup>d</sup> n = 23.
<sup>e</sup> The serum result served as control (x) vs paired heparin-containing specimen results (y) for calculation of linear regression and Student's t-test parameters.

Table 4. Comparison of blood gas measurements of specimens collected with CNLZ heparin with those of specimens collected with EB heparin.

<table>
<thead>
<tr>
<th></th>
<th>CNLZ heparin</th>
<th>EB heparin</th>
<th>Mean</th>
<th>Slope</th>
<th>Intercept</th>
<th>S&lt;sub&gt;xy&lt;/sub&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO&lt;sub&gt;2&lt;/sub&gt;, mmHg</td>
<td>235</td>
<td>241</td>
<td>0.94</td>
<td>9</td>
<td>18</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.43</td>
<td>7.43</td>
<td>0.90</td>
<td>0.75</td>
<td>0.01</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt;CO2&lt;/sub&gt;, mmHg</td>
<td>36</td>
<td>36</td>
<td>0.96</td>
<td>1.6</td>
<td>1.1</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

n = 26.

allowed for occasional delays in analysis, yet would not artifactually alter the iCa concentration of the specimen. The ideal syringe would also be suitable for blood collection for other commonly performed tests. Such versatility reduces the types of specimen containers required, thus minimizing costs (in inventory and maintenance of stocks) and reducing instances in which the use of the wrong syringe for a specific test risks the reporting of erroneous results or requires redraw of specimens. The increasing recognition of the need to reduce iatrogenic blood loss by minimizing specimen volume in certain groups of patients, including the very young and very old and those with leukemia or acquired immunodeficiency syndrome, has increased the numbers of syringe specimens arriving in the clinical laboratory for tests besides blood gas analysis.

Currently available commercial products are far from ideal, particularly with respect to iCa and total calcium analyses. Many hospitals heparinize syringes for collection of iCa specimens by drawing into the syringe a concentrated solution of Li (or Na) heparin and expelling the excess before drawing blood. This rinsing procedure may artifactually reduce iCa concentrations by two mechanisms: (a) binding of iCa by the heparin left in the dead space of the syringe, and (b) dilution of specimen by the heparin solution in the dead space of the syringe (typically 100–200 μL for a 3-mL syringe). As the results of our initial experiment demonstrate (Fig. 1), the degree of artificial change in iCa concentrations depends on the heparin content of the syringe and the volume of the blood draw. Rinsing syringes with highly concentrated heparin solutions results in highly variable (procedure-dependent) and unknown changes in iCa concentrations due to heparin interference. Commercially available syringes containing lyophilized heparin are clearly advantageous, because the possibility of specimen dilution is eliminated and the amount of heparin present is precisely controlled. However, even small amounts of Li heparin interfere in iCa measurements, and the reduction in interference of heparin obtained by decreasing the amount of heparin to the minimum required for anticoagulation may be defeated in instances in which draw volume is reduced to minimize iatrogenic blood loss. At least one manufacturer (Martell Medical Products, Temecula, CA) has sought to eliminate heparin interference in iCa measurements by use of Zn heparin. The studies presented here (Figs. 1 and 2) indicate that even at low doses, Zn heparin artifactually increases iCa con-
centrations, and use of standard doses of Zn heparin (100 units/3-mL syringe) artifactually decreases whole-blood pH.

The use of EB heparin greatly reduces interference in iCa measurements, even when the heparin concentration is increased by partial filling of the syringes (Fig. 1) (8, 9), but total calcium concentrations are significantly increased. Laboratories collecting iCa specimens in EB heparin-containing syringes must require that total calcium specimens drawn from arterial or central lines be collected in an alternative heparin-containing syringe, which inevitably will result in specimens being drawn in the inappropriate syringe. Also, the presence of bound calcium in EB heparin has been reported to “buffer” changes in iCa so that iCa values at the extremes of the pathological range are moderately biased toward normality (9, 12).

CNLZ heparin can reduce or eliminate heparin interference in iCa measurements while preserving the utility of specimens collected for blood gas, total calcium, and other routine laboratory analyses. The rationale behind this product is that (i) heparin interference in iCa measurements occurs as the result of binding of calcium by heparin (5, 13); (ii) heparin is a heterogeneous material containing multiple cation-binding sites with varied affinity for cations (10); (iii) titration of the heparin cation-binding sites with a graded amount of zinc allows chelation of the binding sites with greatest affinity for divalent cations such as zinc or calcium; (iv) zinc ions, once bound to the high-affinity sites in the heparin preparation, prevent calcium binding to these sites; and (v) occupation of the low-affinity cation-binding sites by lithium minimizes unwanted changes in blood gas parameters, since Li heparin is a suitable anticoagulant for blood gas analysis. Initial experiments determined the range of zinc content of CNLZ heparin, which minimized heparin interference in iCa measurements. Though iCa values in specimens collected from volunteers with syringes containing 36 units of lyophilized CNLZ heparin differed slightly but systematically from values obtained in concomitantly collected blood specimens without anticoagulant, this difference averaged 0.01 mmol at both full and partial draw, which is trivial from a clinical standpoint. Blood gas parameters and total calcium results were in close agreement with nonheparinized controls. Similar results were obtained in the study involving specimens from intensive-care-unit patients; iCa values were systematically but trivially greater than values obtained from concomitantly collected serum specimens, as were iCa results for specimens collected with EB heparin.

We conclude from these studies that syringes containing lyophilized CNLZ heparin minimize the interference of heparin in iCa analysis, and thus are suitable for collection of specimens for iCa, blood gas, and electrolyte analysis; such specimens are also free of interference in total calcium determinations.

This work was supported in large part by Sherwood Medical Co. We thank Elizabeth Legwinska for theoretical and technical guidance throughout this study, and Barbara Hartman for assistance in preparation of this manuscript.

References